THE COMBINED EFFECT OF ULTRAVIOLET IRRADIATION AND HEAT UPON CLEAVAGE OF SEA URCHIN EGGS[1]

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The damaging, even lethal, effect of heat on cells following a sublethal dosage of ultraviolet light was first reported by Bovie and Daland (1923), using very short wave-lengths and 30.5° C. upon *Paramecium caudatum*. Reversing the order of the stimuli had much less effect. They called the effect produced sensitization.[2] Curran and Evans (1938) confirmed Bovie and Daland's finding, employing bacterial spores, heat and wave-length 254 millicrons. Giese and Crossman (1945) later reported the same effect in two species of *Paramecium*.

More recently, Garay and Guba (1951) have sensitized adenosine triphosphate to the splitting action of B-myosin; also, Kleczkowski (1954) has reduced the stability of the chymotrypsin molecule and caused an increased rate of denaturation of tobacco mosaic virus through exposure of these molecules to ultraviolet light and elevated temperature.

The significance of the experiments of this paper lies in the finding of an absence of sensitization of *Arbacia* eggs to a temperature of 36° C. through irradiation with wave-length 254 mμ, and the finding of a tendency toward sensitization in *Strongylocentrotus* eggs when ultraviolet light and a temperature of 31.4° C. were used; also, in an examination of the effects which equivalent doses of heat and ultraviolet light, singly, combined and in alternative order, produce in egg protoplasm.

Method

For the *Arbacia* experiments, Westinghouse Sterilamp #WL 782L30, over 91% of whose emission is in wave-lengths 254 mμ, was operated before use for a minimum of 10 minutes to insure constancy of output. Throughout the 9 weeks of experiments the energy liberated was 770 ergs per square millimeter per second, as measured by an ultraviolet light meter which recently had been calibrated. Irradiation, which took place at room temperature, average 24° C., was carried out at a distance of 81 mm. beneath the approximate center of the lamp and in a small circumscribed area. A single layer of eggs, covered by 3 mm. of sea water, was exposed in Syracuse watch glasses. All eggs were concentrated within a 10-mm. radius by rotation of the dishes. The average rise in temperature of the sea water due to heat generated by the ultraviolet lamp was found to be 0.5° C.

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[2] Sensitization: The production of an exaggerated injury after sublethal irradiation by a sublethal dose of some secondary agent, and the absence of such exaggerated injury when the agent and irradiation are given in reverse order.

for 120 seconds and 0.6° C. for 200 seconds. The length of exposure to irradiation in different experiments was 40, 80, 120, 160 or 200 seconds.

For the *Strongylocentrotus purpuratus* experiments with the West Coast sea urchin, two small research ultraviolet lamps with 93% of emission at wave-length 254 mμ were used. These were placed 10 cm. above and below the eggs. Eggs were exposed in a small quartz vessel which held, in a single layer, from 1500 to 2000 eggs. By the use of other vessels, two thicknesses of quartz and 2 ml. of sea water were traversed by the ultraviolet light from above and below the eggs. Each 4-cm., nearly circular lamp, constructed of Vycor tubing 1 cm. in diameter, yielded approximately 25 ergs per square millimeter per second when 15 milliamperes of current passed through each lamp tube (wired in series). By means of a Variac in the circuit, voltage could be varied, which permitted delivery of graduated intensities of ultraviolet light within the adopted time interval of ten seconds. The lamps' output was measured by a Hanovia ultraviolet light meter, recently calibrated for 254 mμ radiations. For the standardized 10-second exposure, 250 ergs per square millimeter reached each of the upper and lower surfaces of every egg, approximately 5000 microwatts per square centimeter.

A water-bath of approximately 40 liters capacity, equipped with a blade stirrer, was maintained by means of a thermostat at 36° ± 0.5° C. for *Arbacia,* and at 31.4° ± 0.5° C. for the *Strongylocentrotus* experiments. Thin glass test tubes, calibrated to hold 4 ml., were filled with fresh sea water from 10 to 20 minutes before use and placed in a rack within the water-bath, ample time to permit the tubes of sea water to equilibrate with the temperature of the water-bath.

Sea urchin eggs were inseminated before use and examined for presence of normal fertilization membranes. All *Arbacia* experiments were begun 10 minutes, and *Strongylocentrotus* experiments from 20 to 30 minutes after insemination, since preliminary experiments had indicated that these were especially sensitive periods. All 6 phases of one complete experiment were performed in many cases upon the same batch of eggs. To secure comparable biological effects with heat and ultraviolet light, selection of the graduations in the intensity of each agent was by comparison of one- and two-day-old larvae that had emerged from treated eggs. Size, defects in spines and rate of swimming were the criteria used in establishing comparable biological effects.

Treatments were paired. For instance (a) 10 seconds' irradiation plus exposure to heat for 1, 2, 3, 4 or 5 minutes and the same two agents employed in reverse order constituted one pair of treatments; (b) two minutes' exposure to heat plus exposure to the ultraviolet radiation generated by 5, 15, 25, 35 and 45 milliamperes of current and these same two agents in reverse order constituted a second pair of treatments; (c) the use of each physical agent by itself—heat and ultraviolet light—constituted the third pair of treatments.

The fertilized sea urchin eggs were concentrated by centrifugation. When irradiation preceded heat, the eggs were diluted 75 times; when heat preceded irradiation, the dilution was 38 times. Both dilutions were sufficient to ensure that as each egg, whose diameter is 75 to 80 μ, sank it was heated almost instantaneously to 36.0° C. (*Arbacia*) or to 31.4° C. (*Strongylocentrotus*). The amount of water introduced with 1/10-ml. suspension of eggs was negligible compared with the 4-ml. volume of receiving medium. After the particular temporally-graduated

dose of heat was given, contents of the heated tube were poured into a Syracuse watch glass that had been rinsed with sea water and stored at 1° C. for at least one-half hour prior to use. Thus, the heating effect was stopped swiftly since contact with the cold dish brought the temperature of the sea water to 3° C. in 180 seconds. Those eggs which required irradiation were then subjected to the appropriate ultraviolet light treatment. Although the sudden exposure to 1° C. served as a slight stimulus, no analysis of this relatively minor agent has been made since it was a constant factor throughout the experiments.

All controls and experimental dishes containing *Arbacia* eggs were kept in dim light upon a table of running sea water, mean temperature 22° C., whose temperature variation during the course of any one experiment averaged ±0.2° C. *Strongylocentrotus* eggs underwent all treatments in the dark and were reared in darkness at a mean temperature of 12.2° C. ± 0.6° C.

The time at which 50% of the sea urchin eggs underwent the first cleavage was determined by observation of the eggs through a stereoscopic microscope and the count was tallied by the use of hand counters. In fields picked at random the number of divisions observed occurring at one certain time per 100 eggs was counted at least twice (estimated error of 0.5%). In dishes containing more severely damaged eggs, the time at which 50% of first cleavage was attained could be and was plotted graphically. Since it was impossible to decide with accuracy when 50% of the second or third cleavages had been reached by the drastically treated eggs, only data regarding first cleavage were accepted. If an examination of second cleavage of the control eggs revealed the presence of more than 5% of abnormal cleavages, the entire batch of control and experimental eggs was discarded as inferior material.

Results and Interpretation

In Figure 1 the essential differences in contour of curves between heat (symbol o) and ultraviolet light (symbol ●) are presented. Note that in the controls the average interval between insemination and attainment of first cleavage by 50% of the eggs is 52 minutes in *Arbacia* and 126 minutes in *Strongylocentrotus*. The *Arbacia* heat curve rises slowly at first, followed by a steep change in slope between gradations 3 and 4, which suggests a chemical reaction of the first order. Although no readings between these gradations have been taken, the curve has been drawn in this region with a minimum deviation from the extrapolated extension of each of its limbs. In the ultraviolet curve a sharp rise occurs in the first 10 seconds. Thereafter, the curve ascends slowly and uniformly. The abrupt change in slope seems to indicate an intense initial ultraviolet action upon sea urchin eggs, followed by a more gentle secondary action. The seemingly straight line of the secondary action of ultraviolet light reveals the proportionality between dosage and effect. If one compares the effect of short exposures of eggs to those two physical agents, ultraviolet light causes a greater delay in the onset of cleavage than heat does. Attention is called to the fact that 10 seconds of ultraviolet light caused the delayed first cleavage to occur 73 minutes after insemination of the *Arbacia* eggs and the second gradation of heat caused cleavage to occur 75 minutes after insemination. Thus, each agent which was used as a secondary stimulus produced an equivalent delay in first cleavage.

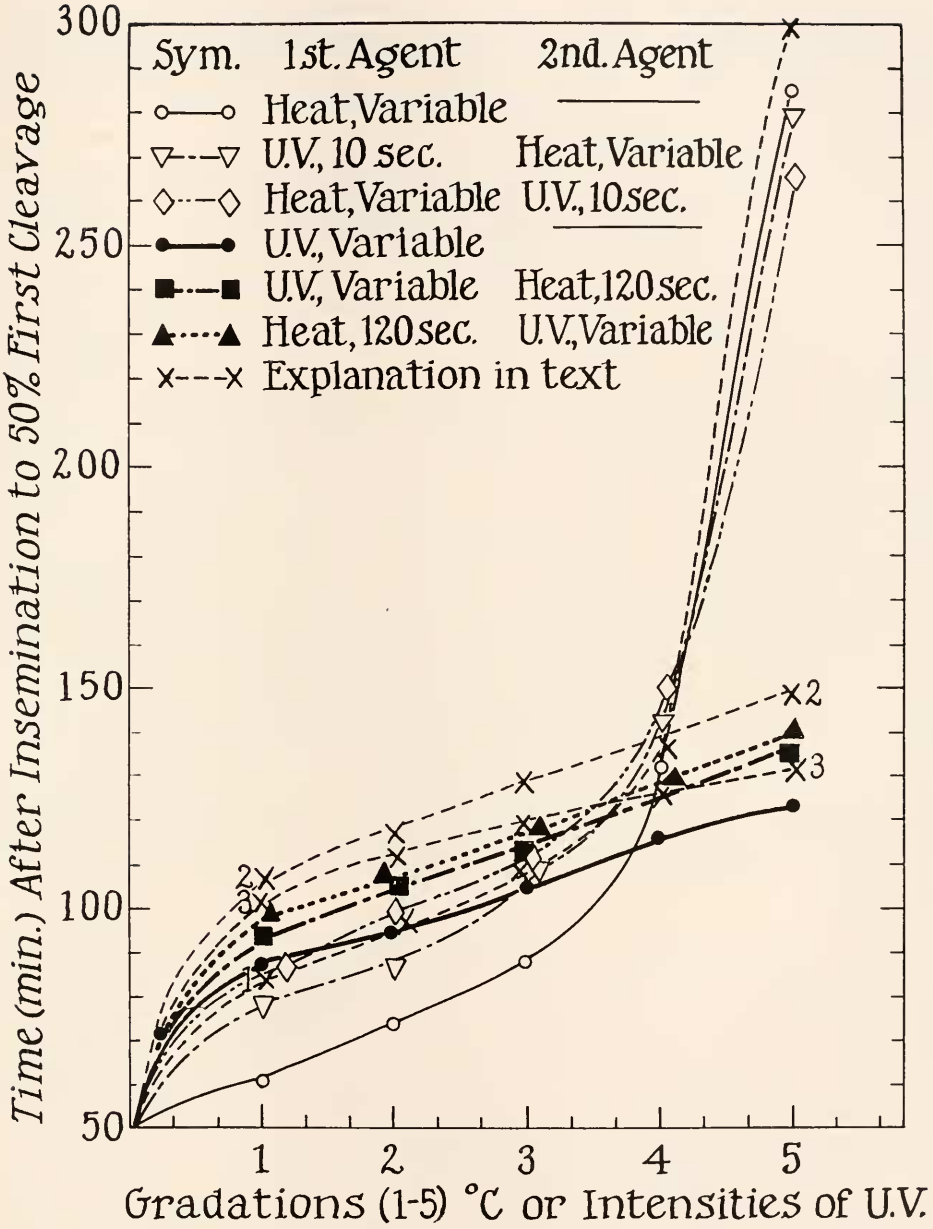

FIGURE 1. *Arbacia* eggs. Basic curves of cleavage delays resulting from exposure to graduated doses of heat or 254 mμ of light. Also, delays caused by combinations of stimuli, applied in the order indicated in the table.

In Figure 1, *Arbacia,* it may readily be observed that the experimental curves represented by solid lines tend to fall into two subfamilies; those in which the heat treatment was given in graded exposures resemble the basic heat curve, and likewise, those in which the ultraviolet treatment was given in graded intensities resemble the basic ultraviolet curve.

Apparently only graduated exposures to ultraviolet irradiation (and heat treatment) produce conditions within a cell which hinder full expression of damage caused by whichever agent is administered secondarily. The 10-second exposure to ultraviolet radiation, which is used as a minor agent in combination with graded exposures of heat, should produce only the initial or intense ultraviolet reaction. Hence, its effect and that of heat should be completely additive. If to the delay produced by heat treatments there is added the 21-minute delay caused by 10 seconds of ultraviolet irradiation, the broken-line curve, numbered 1, is obtained. Since neither curve that represents a combination of graded exposures to heat plus minor ultraviolet radiation differs significantly from the hypothetical curve, numbered 1, the effect of a small dose of ultraviolet light plus exposure to gradations of heat is an additive one. In the curves in which ultraviolet light is given in serial dosages, both initial and successive effects of irradiation are present. Hence, it is reasonable to suppose that if heat and ultraviolet action both were to affect the same cellular constituents, that agent administered after the first one, whether radiant or thermal, would act upon protoplasm already injured by the first agent and could not exert its full retarding effect upon cleavage. Such seems to be the case, for the curves representing graded dosages of ultraviolet light, plus heat as the minor agent, nearly coincide with the broken-line curve numbered 3, and not with the curve numbered 2 which represents a theoretical sum of the delays. The curve numbered 3 represents seven-eighths of the sum of the graded dosages of ultraviolet light and gradation 2 of heat exposure. Experimental results therefore indicate that, except for the briefest of exposures, whenever ultraviolet radiation is employed in graduated doses in combination with a condition of elevated temperature, neither treatment produces its full effect upon the protoplasm of the *Arbacia* egg.

One is forced to conclude that under the conditions of these experiments with *Arbacia* eggs there is absence of sensitization to heat by preliminary irradiation with ultraviolet light of wave-length 254 mμ. Were sensitization present, a heat treatment following irradiation should cause a considerable delay in cleavage over that brought about by the same treatments given in reverse order. But it will be noticed that the two-agent curves of the heat subfamily show no statistically significant differences between them and, likewise, the two-agent curves of the ultraviolet subfamily show only insignificant differences.

However, an examination of the curve representing the effect of treatment of fertilized *Strongylocentrotus* eggs by the fixed 10-second ultraviolet irradiation and graded doses of heat (Fig. 2—symbol $\bigtriangledown$) reveals a strong trend toward sensitization. As indicated by the dash-arrow on curve with symbol $\bigtriangledown$, no batch of eggs achieved the designated criterion of 50% of first cleavage if pretreated for 10 seconds with ultraviolet light followed by gradations 4 or 5 of heat. Counts of cleavages revealed that in only 33% of the experiments did those eggs which received ultraviolet irradiation before any length of exposure to a temperature

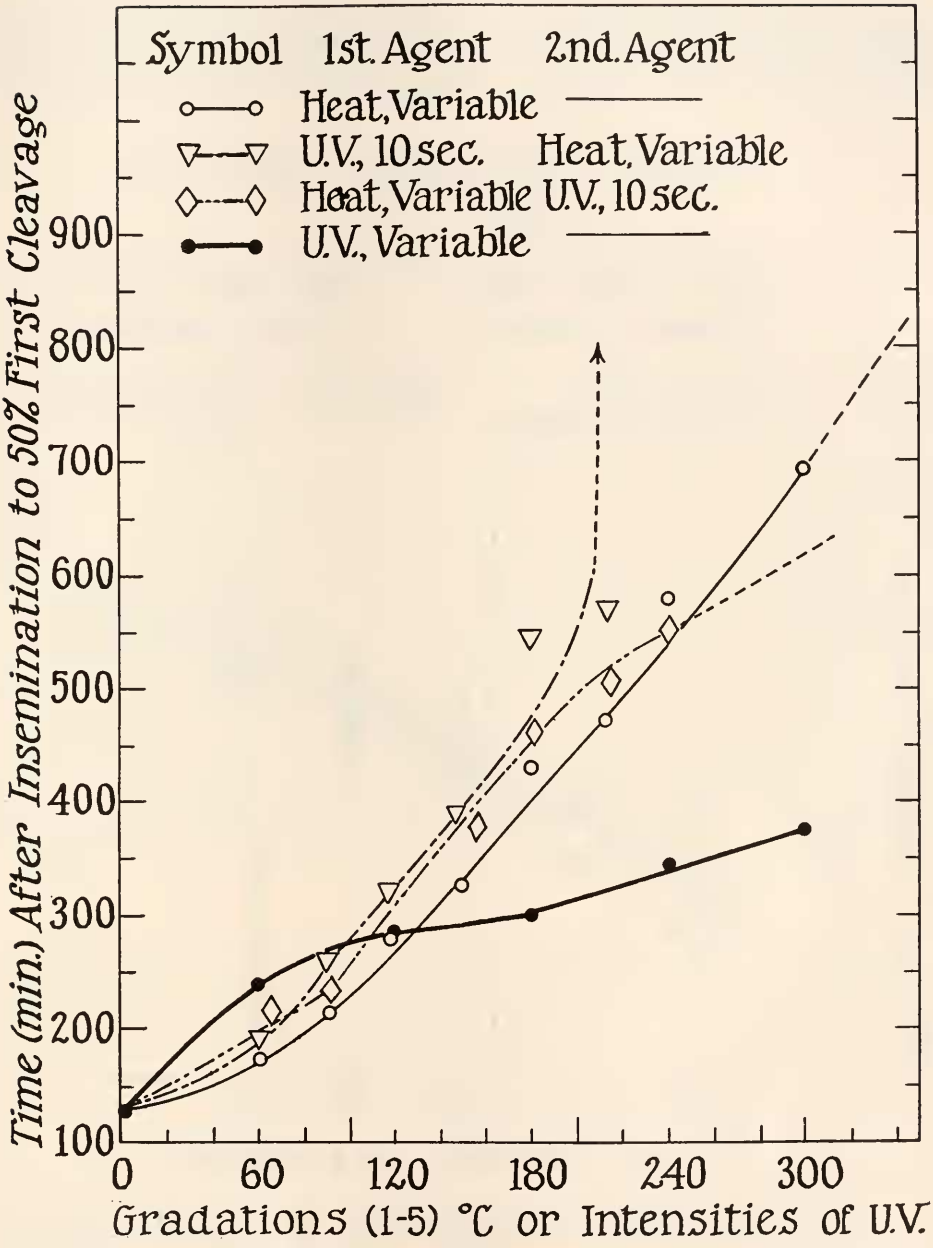

FIGURE 2. *Strongylocentrotus* eggs. Curves representing delays in attaining 50% of first cleavage caused by exposures as indicated in table. Note order of application of stimuli.

of 31.4° ± 0.5° C. reach 50% of first cleavage, whereas among those eggs getting the same dosages but receiving ultraviolet light as the latter treatment nearly all attained the 50% cleavage criterion. Had *Arbacia* eggs been heated at a slightly higher temperature, it is probable that ultraviolet light sensitization to heat would

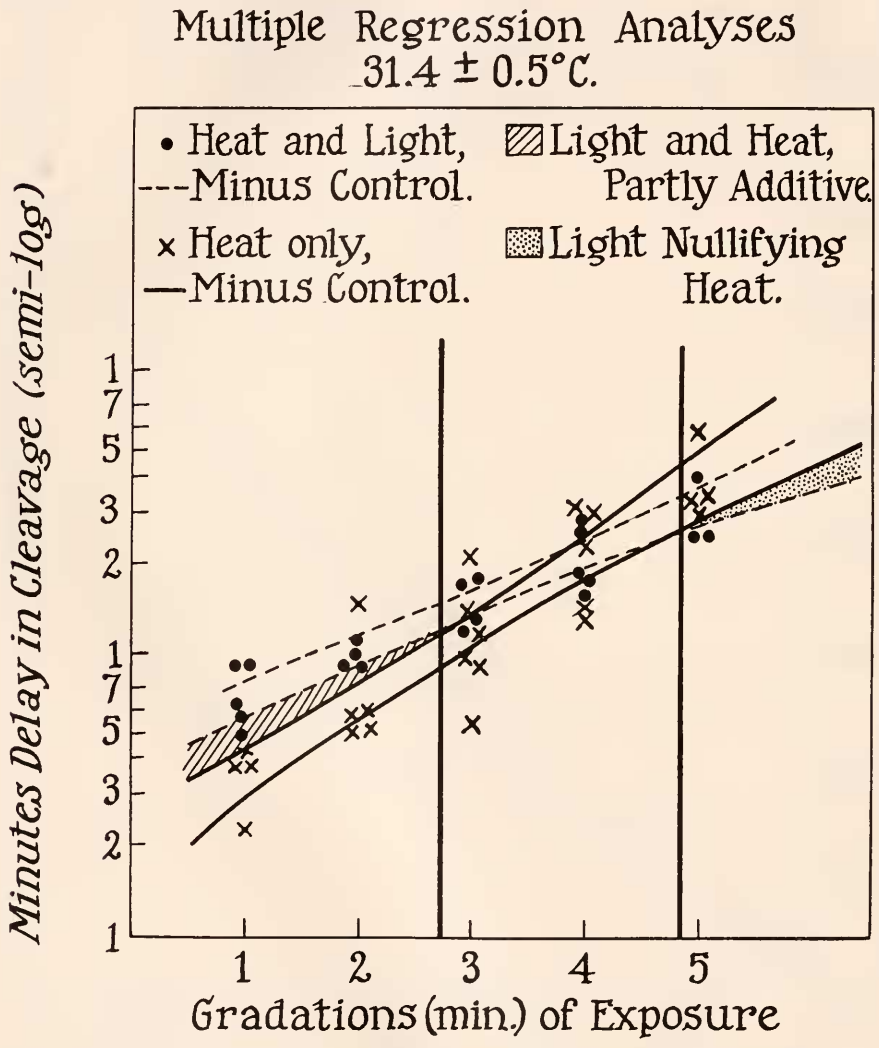

FIGURE 3. Multiple regression analyses of *Strongylocentrotus* data regarding delays in attaining 50% of first cleavage.

have been shown, similar to that revealed by a multiple regression analysis of the above-mentioned *Strongylocentrotus* data. In view of the fact that light treatment alone should produce from 50 to 80 minutes delay in cleavage, it seems likely that the heat-plus-light treatment delayed cleavage at least an hour *less* than would be expected if the heat and light effects were independent and linearly additive. Attention is called, also, to the crossing of the heat-plus-ultraviolet light curve (Fig. 2—symbol ◇) below the heat-only curve at gradation $3\frac{1}{2}$ (230 seconds), a difference which is indicative but not statistically significant.

A semilogarithmic plot of the heat curve of both *Arbacia* and *Strongylocentrotus* yielded two straight lines; only gradation 5 of *Arbacia* did not lie on its exponential curve. The slopes for these lines—*Strongylocentrotus,* 0.166 and *Arbacia,* 0.102—revealed that *Strongylocentrotus* eggs, living customarily at a lower temperature than *Arbacia* eggs, were damaged more than the latter by heat administered in graduated dosages. *Arbacia*'s first cleavage at 52 minutes in contrast to *Strongylocentrotus*' at 126 minutes showed the response to temperature that would be expected of animals reared at 22° C. and 12.5° C., respectively. For this temperature separation of about 10 degrees, the predicted Q_{10} of approximately 2 occurred.

Figure 3 indicates that the regression coefficient for heat-only is significantly greater at the 1% level than the regression coefficient for heat followed by 10 seconds of ultraviolet light. Since *Strongylocentrotus* eggs which have been subjected to both heat (31.4° ± 0.5° C.) and light treatments show significantly less additional delay in achieving 50% of first cleavage as the time of exposure is increased than do those which have undergone only heat exposures, this indicates that the addition of light diminishes the harmful effects of heat when the whole range from 1 to 5 minutes is considered.

That recovery from mild injuries caused by the two physical agents, used singly or combined, does occur could be observed in the large percentage of normal or nearly normal sea urchin embryos and plutei obtained. Survivors from among the more drastically treated eggs revealed evidence of exposure to heat and ultraviolet light by their small size, shortened or missing spines and other blemishes.

Discussion

That the effects of ultraviolet irradiation are multiple (Heinmets and Nathan, 1954) has been brought out clearly by data published in the last decade. Cytolysis, mentioned by Blum, Cook and Loos (1954), the clumping of pigment of the cortex, reabsorption of intercellular boundaries, as well as delays in cleavage and abnormal cleavage patterns, were evident in the experimental material of this paper.

Probably, the most important effect of ultraviolet irradiation is upon nucleic acids. Iverson (1957), using *Tetrahymena pyriformis,* found that DNA synthesis was inhibited by doses of wave-length 254 mμ that permitted a continuation of cellular enlargement, also, that RNA synthesis ceased, following irradiation, after about one division cycle. Since DNA functions in the replication of chromosomes, cleavage could not occur until sea urchin eggs had recovered from insult to nucleotide precursors of DNA.

Novick and Szilard (1949) have postulated the formation in protoplasm of both a stable and a labile poison by treatment with ultraviolet light. The labile poison was considered to be rendered non-toxic by white light. Since it has been shown

that post-treatment of *Strongylocentrotus* eggs with ultraviolet light of the germicidal wave-length, 254 mμ, significantly reduces a delay in attaining first cleavage that was caused by heat, the formation of a stable poison through the action of ultraviolet light seems questionable.

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Summary

1. Thermal treatment (36° C. *Arbacia;* 31.4° C. *Strongylocentrotus*) applied to sea urchin eggs in graduated exposures causes a delay in first cleavage that is roughly proportional to the exposure.

2. Graduated intensities of 254 mμ of ultraviolet light produce a delay in first cleavage that is less pronounced with increasing intensities of light.

3. Treatments involving both ultraviolet irradiation and heat retard the first cleavage of sea urchin eggs more than either agent alone.

4. Curves of cleavage delays of *Arbacia* eggs caused by combined ultraviolet light and 36° C., either one serving as the major physical agent and the second as the minor physical agent, show no statistically significant difference from a hypothetical curve representing a sum of the two single-agent delays.

5. Although there is no evidence that ultraviolet irradiation preceding heat causes an exaggerated injury, which application of the same agents in reverse order fails to do with *Arbacia* eggs, there is an indication that such sensitization tends to occur in *Strongylocentrotus* eggs pretreated with 254 mμ of ultraviolet radiation and then subjected to heat at 31.4° ± 0.5° C.

6. *Strongylocentrotus* eggs pretreated to a temperature of 31.4° C., followed by exposure to ultraviolet irradiation (254 mμ), show significantly *less* delay in achieving first cleavage than eggs subjected only to a temperature of 31.4° C.

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